275-Pos Board B154

Laser Microbeam-induced Spatiotemporal Change in Refractive Index of Chromosomes in Living PTK2 Cells

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Ultra-short laser pulses, which allow for confinement of damage to the focal volume, are used routinely to ablate organelles inside living cells, yet the physical mechanisms of this ablation are not completely understood. Many different parameters, particularly wavelength and laser pulse duration, are used in experiments from different laboratories, but no systematic delineation of threshold energy and irradiance for microsurgery of chromosomes using various wavelengths has been reported.

In a series of comparative experiments using three different lasers - a 200 femtosecond near-infrared, a 12 picosecond green, and a 12 nanosecond green - we measured energy and irradiance thresholds for inducing a change in the refractive index of micro-irradiated regions of chromosomes in mitotic PTK2 (*Potorous tridactylus*) kidney cells. To determine the exact irradiance at the focal point, we used the dual objective method for the wavelengths used in our study. While the required energy per pulse for a detectable change in refractive index was highest for the nanosecond laser, the required peak irradiance was highest for the femtosecond laser. The width of the observed damaged spot varied with pulse duration. The threshold for this change in refractive index was found to depend on the spatial pattern of irradiation - point versus line. Further, for a fixed wavelength, the time required to induce a detectable change in the refractive index decreased with increasing exposure time.

In addition, a quantitative phase imaging system to quantify the damage in the micro-irradiated regions is being developed. This system will allow more precise quantitation of the change in refractive index in the chromosomes following exposure to the different laser parameters.

From these results, specific insights can be gained into the physical mechanisms of laser ablation.

276-Pos Board B155

Photonic and Magnetic Force Micro-piston: An integrated force/microfluidic device for investigating expansion and compression stress in chromatin/chromosomes and their roles in chromosome function.

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Dynamic changes in the state of chromosomes (changes in the level of chromatin compaction) take place in the confined environment of the nucleus throughout the cell cycle. These changes have been hypothesized to determine both the mechanical properties and, as a direct consequence of such effects, the function of chromosomes (Kleckner et al., 2004). To test this hypothesis, we have developed devices that can use either photon pressure or magnetic force to push beads in microfluidic channels, creating compression chambers in which the response of a confined biological specimen can be investigated as a function of force as well as changes in buffer, extract or biochemical composition. Photon pressure is generated by a weakly focused laser beam emanating from a fiber optic mounted directly on to our microfluidic chip, thus eliminating the need for the fixed optics associated with typical laser trapping systems. As a result, heating and the associated risk to a biological specimen is greatly reduced. Magnetic force is generated by a thin foil electromagnet system capable of producing high field and field gradients that, when used in conjuction with a 4.5 micron magnetic bead, can produce hundreds of picoNewtons of compression force on a biological specimen. These devices will be used to address three immediate questions of interest: (i) What is the magnitude of the force generated by chromatin expansion? (ii) When two chromatin masses are present, to what extent do they intermingle? and (iii) When expansion is constrained by confinement, does the resulting compression stress alter the structure and/or properties of chromatin?

277-Pos Board B156

Counterion Induced Electrostatic Condensation Of Nucleosomes And Chromatin Arrays

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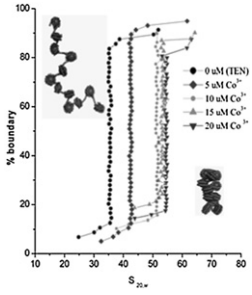
Recombinant molecular biology techniques have enabled preparation of pure and well-defined nucleosome core particles (NCP) and chromatin arrays. The 12_177_601 DNA sequence yields chromatin arrays with 12 histone octamers that can be studied with biophysical methods. The importance of the basic N-terminal histone tails can be investigated. The multivalent salt induced compaction of chromatin arrays and the aggregation of arrays, mononucleosomes (177 bp) and NCPs (147 bp), have been studied and compared with computer modelling using coarse-grained models.

The results show that the critical concentration of multivalent counterion concentration needed for compaction or aggregation, for all three systems follow the order $Na^{+} \approx K^{+} >> Mg^{2+} \approx Ca^{2+} >>$ spermidine^{3+} > $Co(NH_3)_6^{3+}$ > spermine^{4+}. This is in accordance with polyelectrolyte theory and a mechanism of condensation due to salt screening, attractive ion correlation and histone tail bridging, which in theoretical modelling must be described incorporating explicit mobile ions (beyond Debye-Hückel). The figure shows analytical ultracentrifugation sedimentation velocity measurements during titration of the chromatin array with $Co(NH_3)_6^{3+}$ inducing compaction. The inset demonstrates the compaction in coarse-grained computer modelling with explicit $Co(NH_3)_6^{3+}$ ions (right).



278-Pos Board B157

A Course Grain Model of Histones and DNA in the Nucleosome

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There have been significant advances and interest in developing coarse grained models of DNA based on elastic rod theories. These models have focused largely on describing the physical-mechanical behavior of the DNA itself, without protein. Here we utilize coarse-grain models for both DNA and protein molecules to investigate the interaction of DNA with protein-complexes. We have focused our attention on the level of detail required in the coarse graining to achieve large scale deformations of the DNA. For this purpose we have chosen the nucleosome, a histone-DNA complex as our example system because (a) there is significant deformation of DNA in the bound state as compared to the free state (i.e., 1.7 turns of a superhelix), (b) the DNA in the complex is on the order of one persistence length, and (c) there are no sequence specific contacts between the protein and the DNA. These three features perhaps make this system a right candidate to be described by the physical-mechanical behavior of DNA.

In our model of the nucleosome the DNA is represented, numerically, as a continuous elastic rod with a continuous charge distribution interacting with a multipolar representation of the electrostatic potential for the nucleosome's histone core. A contact (non-penetration) constraint is also included to account for the histone excluded volume. Using this model we investigate the physical basis of superhelical formation and the dependency of nucleosome stability on DNA sequence.

279-Pos Board B158

Quantitative Model and Analysis of Nucleosome Organization near the Transcription Start Site in Yeast

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Several recent experiments determined nucleosome positions in the yeast genome [1-3]. These studies robustly identified two salient features in the spatial organization of nucleosomes: (i) nucleosome-free regions upstream of many transcription start sites (TSS), and (ii) an oscillatory nucleosome density downstream of the TSS. However, the mechanisms underlying these patterns are less clear. One possible scenario is that the majority of nucleosome positions near the TSS are directly determined by the DNA sequence [4], binding competition with other proteins [5], or by active remodelling. An alternative scenario is that only a minority of these nucleosomes is directly positioned by the DNA sequence, forming barriers which strongly constrain the positions of closeby nucleosomes, purely on statistical grounds [3]. Specifically, the nucleosomes might be seen as a one-dimensional gas of rods (Tonks gas) with a few barriers in between. We quantitatively test this latter scenario from basic physical principles. To this end, we assess whether the experimentally observed oscillations in nucleosome occupancy are indeed compatible with Tonks gas statistics. Furthermore, we estimate whether biologically reasonable binding specificity suffices to form barriers able to create nucleosome free regions as observed.

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